PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 37/00, A01N 43/16 — A1

(11) International Publication Number: WO 95/11690

(43) International Publication Date: 4 May 1995 (04.05.95)

(21) International Application Number: PCT/US94/12333

(22) International Filing Date: 24 October 1994 (24.10.94)

(30) Priority Data:

143,571	26 October 1993 (26.10.93)	US
168,776	16 December 1993 (16.12.93)	US

(60) Parent Applications or Grants
(63) Related by Continuation

US	143,571 (CIP)
Filed on	26 October 1993 (26.10.93)
US	186,776 (CIP)
Filed on	16 December 1993 (16.12.93)

(71) Applicant *(for all designated States except US)*: EMISPHERE TECHNOLOGIES, INC. [US/US]; 15 Skyline Drive, Hawthorne, NY 10532 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MILSTEIN, Sam, J. [US/US]; 1 Knollwood Drive, Larchmont, NY 10538 (US). BARANTSEVITCH, Evgueni, N. [RU/US]; Apartment 2B, 127 Cooper Drive, New Rochelle, NY 10801 (US).

(74) Agents: ROBINSON, Joseph, R. et al.; Darby & Darby P.C., 805 Third Avenue, New York, NY 10022 (US).

(81) Designated States: AM, AT, AU, BB, BG, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: DESFERRIOXAMINE ORAL DELIVERY SYSTEM

(57) Abstract

Modified amino acids and methods for their preparation and use as oral delivery systems for pharmaceutical agents are described. The modified amino acids are preparable by reacting single amino acids or mixtures of two or more kinds of amino acids with an amino modifying agent such as benzene sulfonyl chloride, benzoyl chloride, and hippuryl chloride. The modified amino acids may form encapsulating microspheres in the presence of the active agent under sphere-forming conditions. Alternatively, the modified amino acids may be used as a carrier by simply mixing the amino acids with the active agent. The preferred acylated amino acid carrier is salicyloyl-phenylalanine. The modified amino acids are particularly useful in delivering biologically active agents, e.g., desferrioxamine, insulin or cromolyn sodium, or other agents which are sensitive to the denaturing conditions of the gastrointestinal tract.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

DESFERRIOXAMINE ORAL DELIVERY SYSTEM

This application is a continuation-in-part of U.S. application serial no. 08/186,776, filed December 16, 1993, which is a continuation-in-part of U.S. application serial no. 08/051,019, filed April 22, 1993, and of U.S. application serial no. 08/143,571, filed October 26, 1993, which is a continuation-in-part of U.S. application serial no. 08/076,803, filed June 14, 1993, which is a continuation-in-part of U.S. application serial no. 07/920,346, filed July 27, 1992, which is a continuation-in-part of U.S. application serial no. 07/898,909, filed June 15, 1992.

Field of the Invention

This invention relates to an oral delivery system, and in particular to modified amino acids for use as a delivery system for desferrioxamine, insulin and cromolyn sodium. The modified amino acids releasably encapsulate active agents and are suitable for oral administration to mammals. Methods for the preparation of such amino acids are also disclosed.

Background of the Invention

Conventional means for delivering pharmaceutical and therapeutic agents to mammals often are severely limited by chemical or physical barriers or both, which are imposed by the body. Oral delivery of many biologically-active agents would be the route of choice if not for the presence of chemical and physicochemical barriers such as extreme pH in the gut, exposure to powerful digestive enzymes, and

impermeability of gastrointestinal membranes to the active ingredient. Among the numerous pharmacological agents which are known to be unsuitable for oral administration are biologically active peptides and proteins, such as insulin. These agents are rapidly destroyed in the gut by acid hydrolysis and/or by proteolytic enzymes.

Prior methods for orally administering vulnerable pharmacological agents have relied on co-administration of adjuvants (e.g. resorcinols and non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether) to artificially increase the permeability of the intestinal walls; and co-administration of enzymatic inhibitors (e.g. pancreatic trypsin inhibitor, diisopropylfluorophosphate (DFF) and trasylol) to avoid enzymatic degradation. Liposomes as drug delivery systems for insulin and heparin have also been described. See, for instance, U.S. Patent No. 4,239,754; Patel et al. (1976) FEBS Letters Vol. 62, page 60; and Hashimoto et al. (1979) Endocrinol. Japan, Vol. 26, page 337. The broader use of the aforementioned methods, however, as drug delivery systems are precluded for reasons which include: (1) the use of toxic amounts of adjuvants or inhibitors; (2) narrow range of low MW cargoes; (3) poor stability of the system and inadequate shelf life; (4) difficulty in manufacturing; and (5) the failure of the method to adequately protect the active ingredient or promote its absorption.

More recently, artificial amino acid polymers or proteinoids forming microspheres have been described for encapsulating pharmaceuticals. For example, U.S. Patent No. 4,925,673 (the '673 patent), the disclosure of which is hereby incorporated by reference in its entirety, describes such microsphere constructs as well as methods for their preparation and use. The proteinoid microspheres of the '673 patent are useful for encapsulating a number of active agents, however the preparation methods result in a complex mixture of high molecular weight (MW) (> 1000 daltons) and low MW (≤ 1000 daltons) peptide-like polymers which are difficult to separate and yield relatively small amounts of the low MW microsphere-forming fraction. Thus, there is a

need in the art for a simple and inexpensive delivery system which is simple to prepare and which can encapsulate a broad range of active agents such as proteinaceous drugs.

Summary of the Invention

Compositions for orally delivering biologically-active agents incorporating modified amino acids, amino acid derivatives, peptides and peptide derivatives as carriers are provided. These compositions comprise

(a) a biologically active agent selected from the group consisting of desferrioxamine, insulin and cromolyn sodium (sodium or disodium cromoglycate);

(b) and an acylated amino acid carrier.

Also contemplated is a method for preparing these compositions which comprises mixing at least one biologically active agent with at least one carrier as described above and, optionally, a dosing vehicle.

In an alternative embodiment, these non-toxic carriers are orally administered to animals as part of a delivery system by blending or mixing the carriers with the biologically active agent prior to administration. The carriers may also form microspheres in the presence of the active agent. The microspheres containing the active agent are then orally administered. Also contemplated by the present invention are dosage unit forms that include these compositions.

According to the invention, modified amino acids are prepared by reacting single amino acids or mixture of two or more kinds of amino acids with an acylating or sulfonating agent which reacts with free amino moieties present in the amino acids to form amides or sulfonamides, respectively. The modified amino acids are then recovered from the mixture.

The modified amino acids are non-toxic and can be orally administered to mammals as a drug delivery system by simply mixing the modified amino acids with an active agent prior to administration. Alternatively, the modified amino

acid may converted into microspheres in the presence of the active agent. The microspheres, containing encapsulated active agent, are then orally administered.

Description of the Drawings

Figure 1 illustrates rat serum calcium levels after oral administration of two dosage levels of a modified amino acid microsphere preparation containing calcitonin encapsulate and soluble preparation containing modified amino acid carrier and calcitonin after pre-dosing with a sodium bicarbonate solution as described in Example 5.

Figure 2 illustrates the induction of iron excretion in Cebus monkeys following oral administration of encapsulated versus unencapsulated desferrioxamine (DFO).

Figure 3 shows the iron excretion in rats following oral administration of encapsulated DFO.

Figure 4 to Figure 8 are graphic illustrations of the results of oral gavage testing in rats with insulin and salicyloyl phenylalanine carrier.

Figure 9 is a graphic illustration of the results of intraduodenal injection testing in rats with insulin and salicyloyl phenylalanine carrier.

Figures 10 and 11 are graphic illustrations of the results of oral gavage testing in rats with disodium cromoglycate and salicyloyl phenylalanine carrier.

Detailed Description of the Invention

All patents, patent applications, and literatures cited in the specification are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure, including definitions, will prevail.

The present invention arose from the discovery that amino acids, in modified form, may be used to orally deliver sensitive bioactive agents, e.g. peptide hormones such as insulin and polysaccharides such as heparin, which would not be considered orally administrable due to their sensitivity to the denaturing conditions of the

gastrointestinal (GI) tract. The modified amino acids may be used as a carrier by mixing it with the active agent or may be converted into microspheres containing encapsulated bioactive agent. In contrast to the modified amino acids of the invention, unmodified free amino acids provide inadequate protective effect for labile bioactive agents against degradation in the GI tract.

Pharmaceutical compositions containing insulin are useful for mammals suffering with diabetes.

Pharmaceutical compositions containing sodium cromolyn, an antiallergic, are useful for mammals suffering from respiratory afflictions, such as asthma or hay fever.

Other advantages provided by the present invention include the use of readily available and inexpensive starting materials and a cost-effective method for preparing and isolating modified amino acids which is simple to perform and is amenable to industrial scale-up production.

The modified amino acids of the present invention may be prepared by reacting single amino acids, mixtures of two or more kinds of amino acids, or amino acid esters with an amine modifying agent which reacts with free amino moieties present in the amino acids to form amides or sulfonamides. Amino acids and amino acid esters are readily available from a number of commercial sources such as Aldrich Chemical Co. (Milwaukee, WI, USA); Sigma Chemical Co. (St. Louis, MO, USA); and Fluka Chemical Corp. (Ronkonkoma, NY, USA).

In practicing the invention, the amino acids are dissolved in aqueous alkaline solution of a metal hydroxide, e.g., sodium or potassium hydroxide, and heated at a temperature ranging between about 5°C and about 70°C, preferably between about 10°C and about 40°C, for a period ranging between about 1 hour and about 4 hours, preferably about 2.5 hours. The amount of alkali employed per equivalent of NH_2 groups in the amino acids generally ranges between about 1.25 and about 3 mmole, preferably between about 1.5 and about 2.25 mmole per equivalent of NH_2. The pH

of the solution generally ranges between about 8 and about 13, preferably ranging between about 10 and about 12.

Thereafter, an amino modifying agent is then added to the amino acid solution while stirring. The temperature of the mixture is maintained at a temperature generally ranging between about 5°C and about 70°C, preferably between about 10°C and about 40°C, for a period ranging between about 1 and about 4 hours. The amount of amino modifying agent employed in relation to the quantity of amino acids is based on the moles of total free NH_2 in the amino acids. In general, the amino modifying agent is employed in an amount ranging between about 0.5 and about 2.5 mole equivalents, preferably between about 0.75 and about 1.25 equivalents, per molar equivalent of total NH_2 groups in the amino acids.

Suitable, but non-limiting, examples of amino modifying agents useful in practicing the present invention include sulfonating agents such as benzene sulfonyl chloride and acylating agents such as benzoyl chloride, hippuryl chloride, salicyloyl chloride and carbodiimide derivatives of amino acids, particularly hydrophobic amino acids such as phenylalanine, tryptophan, and tyrosine.

The reaction is quenched by adjusting the pH of the mixture with a suitable acid, e.g., concentrated hydrochloric acid, until the pH reaches between about 2 and about 3. The mixture separates on standing at room temperature to form a transparent upper layer and a white or off-white precipitate. The upper layer is discarded and modified amino acids are collected from the lower layer by filtration or decantation. The crude modified amino acids are then dissolved in water at a pH ranging between about 9 and about 13, preferably between about 11 and about 13. Insoluble materials are removed by filtration and the filtrate is dried in vacuo. The yield of modified amino acids generally ranges between about 30 and about 60%, and usually about 45%.

If desired, amino acid esters, e.g. methyl or ethyl esters of amino acids, may be used to prepare the modified amino acids of the invention. The amino acid

esters, dissolved in a suitable organic solvent such as dimethylformamide or pyridine, are reacted with the amino modifying agent at a temperature ranging between about 5°C and about 70°C, preferably about 25°C, for a period ranging between about 7 and about 24 hours. The amount of amino modifying agents used relative to the amino acid esters are the same as described above for amino acids.

Thereafter, the reaction solvent is removed under negative pressure and the ester functionality is removed by hydrolyzing the modified amino acid ester with a suitable alkaline solution, e.g. 1N sodium hydroxide, at a temperature ranging between about 50°C and about 80°C, preferably about 70°C, for a period of time sufficient to hydrolyze off the ester group and form the modified amino acid having a free carboxyl group. The hydrolysis mixture is then cooled to room temperature and acidified, e.g. aqueous 25% hydrochloric acid solution, to a pH ranging between about 2 and about 2.5. The modified amino acid precipitates out of solution and is recovered by conventional means such as filtration or decantation.

The modified amino acids may be purified by recrystallization or by fractionation on solid column supports. Suitable recrystallization solvent systems include acetonitrile, methanol and tetrahydrofuran. Fractionation may be performed on a suitable solid column supports such as alumina, using methanol/n-propanol mixtures as the mobile phase; reverse phase column supports using trifluoroacetic acid/acetonitrile mixtures as the mobile phase; and ion exchange chromatography using water as the mobile phase. When anion exchange chromatography is performed, a subsequent 0-500 mM sodium chloride gradient is employed. The modified amino acids may also be purified by extraction with a lower alcohol such as methanol, butanol, or isopropanol to remove low molecular weight non-sphere making material.

The modified amino acids of the present invention are soluble in alkaline aqueous solution ($pH \geq 9.0$); partially soluble in ethanol, n-butanol and 1:1 (v/v)

toluene/ethanol solution and insoluble in neutral water. The titratable functional groups remaining in the modified amino acids are as follows: carboxylic acid groups (COOH) generally ranging between about 1.5 and about 3.5 milliequivalents/g, preferably about 2.3 milliequivalents/g; and amino groups (NH_2) generally ranging between about 0.3 and about 0.9 milliequivalents/g, preferably about 0.5 milliequivalents/g.

If desired, mixtures of amino acids may be used in practicing the invention. Suitable amino acid mixtures include synthetic mixtures of two or more kinds of amino acids and readily available acid or enzyme hydrolyzed vegetable proteins. Sources of hydrolyzed liquid vegetable protein include Ajinomoto USA, Inc. (Teaneck, NJ 07666, USA); Central Soya Co., Inc. (Fort Wayne, IN, USA); and Champlain Industries, Inc. (Clifton, NJ, USA) and additional companies listed in "Food Engineering Master", an annual publication of Chilton Co., Radnor, PA 19089, USA. Prior to modification, the hydrolyzed protein solution is dried and the amino acid mixture is extracted from dried residue with a suitable organic solvent, e.g., methanol or tetrahydrofuran, followed by evaporating the solvent extract.

A particularly preferred hydrolyzed vegetable protein for use in practicing this invention is available from Ajinomoto USA under the tradename AJI-EKI. This product is an acid hydrolyzed liquid soybean protein which is derived from defatted soybean meal and generally contains titratable carboxylic acid groups (COOH) ranging between about 3 and about 8 milliequivalents/g preferably between about 4 and about 6 milliequivalents/g, total free amino groups (NH_2) ranging between about 3 and about 9 milliequivalents/g, preferably ranging between about 4 and about 6 milliequivalents/g NH_2. The molecular weight of the vegetable protein ranges between about 100 D and about 2000 D, preferably between about 100 and about 500 D. The modified amino acids of the present invention are stable and can stored for future use.

The modified amino acids may be used as a drug delivery carrier by simply mixing the modified amino acids with the active ingredient prior to administration. Alternatively, the modified amino acids may be used to form encapsulating microspheres containing the active agent. The modified amino acids of the invention are particularly useful for the oral administration of certain pharmacological agents, e.g., small peptide hormones, which, by themselves, pass slowly or not at all through the gastrointestinal mucosa and/or are susceptible to chemical cleavage by acids and enzymes in the gastrointestinal tract. Non-limiting examples of such agents include human or bovine growth hormone, interferon and interleukin-II, calcitonin, atrial naturetic factor, antigens and monoclonal antibodies.

If the modified amino acids are used as a carrier for an active ingredient, an aqueous solution of modified amino acids is mixed with an aqueous solution of the active ingredient just prior to administration. The solutions may contain additives such as phosphate buffer salts, citric acid, acetic acid, gelatin and gum acacia.

A solution of the modified amino acids is prepared by mixing the amino acids in aqueous solution in an amount ranging between about 1 mg and about 2000 mg, preferably ranging between about 300 mg and about 800 mg per mL of solution. The mixture is then heated to a temperature ranging between about 20 and about 50 C, preferably about 40 C, until the modified amino acid is dissolved. The final solution contains between about 1 mg and about 2000 mg of modified amino acids per mL of solution, preferably between about 300 and about 800 mg per mL. The concentration of active agent in the final solution varies and is dependent on the required dosage for treatment.

The modified amino acids may be used to prepare microspheres for encapsulating active agents. A useful procedure is as follows: Modified amino acids are dissolved in deionized water at a concentration ranging between about 75 and about 200 mg/ml, preferably about 100 mg/ml at a temperature between about 25 C and about 60 C, preferably

about 40 C. Particulate matter remaining in the solution may be removed by conventional means such as gravity filtration over filter paper.

Thereafter, the amino acid solution, maintained at a temperature of about 40 C, is mixed 1:1 (V/V) with an aqueous acid solution (also at about 40 C) having an acid concentration ranging between about 0.05 N and about 2 N, preferably about 1.7 N. The resulting mixture is further incubated at 40 C for a period of time effective for microsphere formation as observed by light microscopy. In practicing this invention, the preferred order of addition is to add the amino acid solution to the aqueous acid solution.

Suitable acids include any acid which does not (a) adversely effect the modified amino acids, e.g., chemical decomposition; (b) interfere with microsphere formation; (c) interfere with microsphere encapsulation of cargo; and (d) adversely interact with the cargo. Preferred acids for use in this invention include acetic acid, citric acid, hydrochloric acid, phosphoric acid, malic acid and maleic acid.

In practicing the invention, a microsphere stabilizing additive preferably incorporated into the aqueous acid solution or into the amino acid solution, prior to the microsphere formation process. The presence of such additives promotes the stability and dispersibility of the microspheres in solution.

The additives may be employed at a concentration ranging between about 0.1 and 5 % (W/V), preferably about 0.5 % (W/V). Suitable, but non-limiting, examples of microsphere stabilizing additives include gum acacia, gelatin, polyethylene glycol, and polylysine.

Under these conditions, the modified amino acid molecules form hollow microspheres of less than 10 microns in diameter. If the modified amino acid microspheres are formed in the presence of a soluble material, e.g., a pharmaceutical agent in the aforementioned aqueous acid solution, this material will be encapsulated in the hollows

of the microspheres and confined within the amino acid wall defined by the spherical structure. In this way, one can encapsulate pharmacologically active materials such as peptides, proteins, and polysaccharides as well as charged organic molecules, e.g., antimicrobial agents, having poor bioavailability by the oral route. The amount of pharmaceutical agent which may be encapsulated by the microsphere is dependent on a number of factors which include the concentration of agent in the encapsulating solution, as well as the affinity of the cargo for the carrier.

The modified amino acid microspheres of the invention are pharmacologically harmless and do not alter the physiological and biological properties of the active agent. Furthermore, the encapsulation process does not alter the pharmacological properties of the active agent. While any pharmacological agent can be encapsulated within the amino acid microspheres, it is particularly valuable for delivering chemical or biological agents which otherwise would be destroyed or rendered less effective by conditions encountered within the body of the mammal to which it is administered, before the microsphere reaches its target zone (i.e., the area in which the contents of the microsphere are to be released) and which are poorly absorbed in the gastrointestinal tract.

The particle size of the microsphere plays an important role in determining release of the active agent in the targeted area of the gastrointestinal tract. Microspheres having diameters between about $\leq$ 0.1 microns and about 10 microns, preferably between about 5.0 microns and about 0.1 microns, and encapsulating active agents are sufficiently small to effectively release the active agent at the targeted area within the gastrointestinal tract. Small microspheres can also be administered parenterally by being suspended in an appropriate carrier fluid (e.g., isotonic saline) and injected into the circulatory system or subcutaneously. The mode of administration selected will, of course, vary, depending upon the requirement of the

active agent being administered. Large amino acid microspheres (>10 microns) tend to be less effective as oral delivery systems.

The size of the microspheres formed by contacting modified amino acid with water or an aqueous solution containing active agents can be controlled by manipulating a variety of physical or chemical parameters, such as the pH, osmolarity or ionic strength of the encapsulating solution, and by the choice of acid used in the encapsulating process.

The amino acid microspheres of the invention may be orally administered alone as solids in the form of tablets, pellets, capsules, and granulates suitable for suspension in liquids such as water or edible oils. Similarly, the microspheres can be formulated into a composition containing one or more physiologically compatible carriers or excipients, and which can be administered via the oral route. These compositions may contain conventional ingredients such as gelatin, polyvinylpyrrolidone and fillers such as starch and methyl cellulose. Alternatively, small microspheres (size less than 10 μm) can be administered via the parenteral route.

The amount of active agent in the composition typically is a pharmacologically or biologically effective amount. However, the amount can be less than a pharmacologically or biologically effective amount when the composition is used in a dosage unit form, such as a capsule, a tablet or a liquid, because the dosage unit form may contain a multiplicity of carrier/biologically-active agent compositions or may contain a divided pharmacologically or biologically effective amount. The total effective amounts will be administered by cumulative units containing in total pharmacologically or biologically active amounts of biologically-active agent.

The total amount of biologically-active agent to be used can be determined by those skilled in the art. However, it has surprisingly been found that with certain biologically-active agents, the use of the presently disclosed carriers provides extremely efficient delivery.

Therefore, lower amounts of biologically-active agent than those used in prior dosage unit forms or delivery systems can be administered to the subject, while still achieving the same blood levels and therapeutic effects.

The amount of carrier in the present composition is a delivery effective amount and can be determined for any particular carrier or biologically-active agent by methods known to those skilled in the art.

Dosage unit forms can also include any of excipients; diluents; disintegrants; lubricants; plasticizers; colorants; and dosing vehicles, including, but not limited to water, 1,2-propane diol, ethanol, olive oil, or any combination thereof.

Administration of the present compositions or dosage unit forms is oral or by intraduodenal injection.

The following examples are illustrative of the invention but are not intended to limit the scope of the invention.

EXAMPLE 1: Modification of Amino Acids with Benzene Sulfonylchloride

A mixture of sixteen amino acids were prepared prior to chemical modification. The constituents of the mixture are summarized in Table 1. 65 grams of the amino acid mixture (total concentration of [-NH_2] groups = 0.61 moles) was dissolved in 760 mL of 1N sodium hydroxide solution (0.7625 equivalents) at room temperature. After stirring for 20 minutes, benzene sulfonylchloride (78 ml, 1 equivalent) was added over a 20 minute period. The reaction mixture was then stirred for 2.5 hours, without heating. As some precipitation had occurred, additional NaOH solution (2N) was added to the solution until it reached pH 9.3. The reaction mixture stirred overnight at room temperature. Thereafter, the mixture was acidified using dilute hydrochloric acid (38%, 1:4) and a cream colored material precipitated out. The resulting precipitate was isolated by decantation and dissolved in sodium hydroxide (2N). This

solution was then reduced *in vacuo* to give a yellow solid, which was dried on the lyophilizer (34.5 g).

TABLE 1: Amino Acid Composition

Amino Acid	Weight (g)	% of Total Weight	No. of moles of each Amino Acid (x10-2)	No. of Moles of -[-NH_2]
Thr	2.47	3.8	2.07	2.07
Ser	2.25	3.46	2.1	2.1
Ala	4.61	7.1	5.17	5.17
Val	4.39	6.76	3.75	3.75
Met	0.53	0.82	0.35	0.35
Ile	2.47	3.8	0.36	0.36
Leu	3.86	5.94	2.95	2.95
Tyr	1.03	1.58	0.56	0.56
Phe	4.39	6.76	0.27	0.27
His	2.47	3.8	1.6	3.2
Lys	4.94	7.6	3.4	6.8
Arg	5.13	7.9	2.95	5.90
Glutamine	9.87	15.18	6.76	13.42
Glutamic Acid	9.87	15.18	6.70	6.70
Asparagine	3.32	5.11	2.51	5.02
Aspartic Acid	3.32	5.11	2.50	2.50

EXAMPLE 2: Preparation Modified Amino Acid/ Salmon Calcitonin Compositions

(a) Preparation of Modified Amino acid microspheres containing encapsulated Salmon Calcitonin

The modified amino acid mixture, prepared in accordance with Example 1, was dissolved at 40°C in distilled water (pH 7.2) at a concentration of 100 mg/ml. The solution was then filtered with a 0.2 micron filter and the temperature was maintained at 40°C. Salmon calcitonin (Sandoz Corp., Basil, Switzerland) was dissolved in an aqueous solution of

to 40°C. The two heated solutions were then mixed 1:1 (v/v). The resulting microsphere suspension was then filtered with glass wool and centrifuged for 50 minutes at 1000 g. The pellet was resuspended with 0.85N citric acid to a volume 5 to 7 fold less than the original volume. Salmon calcitonin concentration of the resuspended pellet was determined by HPLC. Additional microspheres were made according to the above procedure without salmon calcitonin. These "empty microspheres" were used to dilute the encapsulated salmon calcitonin microsphere preparation to a final dosing suspension for animal testing.

(b) Preparation of a Soluble Modified Amino acid carrier/Salmon Calcitonin system

A soluble amino acid dosing preparation containing salmon calcitonin was prepared by dissolving the modified amino acid material in distilled water (pH 8) to an appropriate concentration. The solution was heated to 40°C and then filtered with a 0.2 micron filter. Salmon calcitonin, also dissolved in distilled water, was then added to the modified amino acid solution prior to oral administration.

EXAMPLE 3: *In Vivo* Evaluation of Calcitonin Preparations in Rats

In vivo evaluation of modified amino acid microspheres containing encapsulated calcitonin and soluble modified amino acid carrier/calcitonin system, prepared as described in Example 2, were evaluated in rats. Rats were gavaged with the oral dosing preparations and blood samples were withdrawn at various time intervals for serum calcium concentration determinations.

Nine rats are divided into three groups as follows:

1. calcitonin microspheres: 10 ug calcitonin/kg body weight by oral gavage (3 rats);
2. calcitonin microspheres: 30 ug calcitonin/kg body weight by oral gavage (3 rats); and

3. soluble (unencapsulated) modified amino acid/calcitonin system: 30 ug calcitonin/kg body weight by oral gavage (3 rats). The rats were pre-dosed with 0.7 meq of aqueous sodium bicarbonate solution prior to administration of the soluble system.

Oral gavage dosing of rats is performed. Calcitonin microspheres are prepared immediately prior to dosing and Group 1 rats and Group 2 rats each receive an appropriate dosage of the microsphere suspension. Group 3 rats receives the unencapsulated calcitonin/modified amino acid system. Approximately 0.5 ml of blood is withdrawn from each rat just prior to dosing ("0" time) and 1 h, 2 h and 3 h post-dosing. Serum from the blood samples are stored at -20°C.

The calcium levels of thawed serum taken from group 1-3 rats are analyzed by conventional methods. Experimental results in rats have demonstrated a significant increase in pharmacological activity (i.e., decreasing serum calcium levels) when calcitonin is orally administered either as a encapsulate in modified amino acid microspheres or a mixture with modified amino acids as compared to basal levels. As shown in Figure 1, soluble modified amino acid solution containing salmon calcitonin demonstrated a significant increase in pharmacological activity (i.e., decreasing serum calcium levels) when compared to basal levels after oral administration. The results show that orally administered calcitonin exerted a relatively greater biological effect when administered in its unencapsulated form with modified amino acid as carrier.

EXAMPLE 4: Modification of a Mixture of Five Amino Acids Using Benzene Sulfonyl Chloride

An 86.1 g (0.85 moles of NH_2) mixture of amino acids (see Table 1) was dissolved in 643 mL (1.5 equiv.) of aqueous 2N sodium hydroxide solution. After stirring for 30 minutes at room temperature, benzene sulfonyl chloride (108 mL, 0.86 moles) was added portionwise into the amino acid solution over a 15 minute period. After stirring for 2.5

hours at room temperature, the pH of the reaction mixture (pH 5) was adjusted to pH 9 with additional 2N sodium hydroxide solution. The reaction mixture stirred overnight at room temperature. Thereafter, the pH of the reaction mixture was adjusted to pH 2.5 by addition of aqueous hydrochloric acid solution (4:1, H_2O:HCl) and a precipitate of modified amino acids formed. The upper layer was discarded and the resulting yellow precipitate was isolated by decantation, washed with water and dissolved in 2N sodium hydroxide (2N). The solution was reduced in vacuo to give a yellow solid which was lyophilized overnight. The yield of crude modified amino acid was 137.9 g.

Table 2

Amino Acid	Moles of Amino Acid ($\times 10^{-2}$)	Moles of $[-NH_2]\times10^{-2}$
Valine	7.5	7.5
Leucine	10.7	10.5
Phenylalanine	13.4	13.4
Lysine	21.0	42.0
Arginine	6.0	12.0

EXAMPLE 5: Modification of a Mixture of Five Amino Acids Using Benzoyl Chloride

An 86 g (0.85 moles of NH_2) mixture of amino acids (see Table 2 in Example 4) was dissolved in 637 mL (1.5 equiv.) of aqueous 2N sodium hydroxide solution. After stirring for 10 minutes at room temperature, benzoyl chloride (99 mL, 0.85 moles) was added portionwise into the amino acid solution over a 10 minute period. After stirring for 2.5 hours at room temperature, the pH of the reaction mixture (pH 12) was adjusted to pH 2.5 using dilute hydrochloric acid (4:1, H_2O:HCl). The reaction mixture stirred overnight at room temperature. Thereafter, the pH of the reaction mixture was adjusted to pH 2.5 by addition of aqueous hydrochloric acid solution (4:1, H_2O:HCl) and a precipitate of modified amino acids formed. After settling

for 1 hour, the resulting precipitate was isolated by decantation, washed with water and dissolved in sodium hydroxide (2N). This solution was then reduced in vacuo to give crude modified amino acids as a white solid (220.5 g).

EXAMPLE 6: Modification of L-Valine Using Benzene Sulfonyl Chloride

L-Valine (50 g, 0.43 mol) was dissolved in 376 mL (1.75 equiv.) of aqueous 2N sodium hydroxide by stirring at room temperature for 10 minutes. Benzene sulfonyl chloride (48.7 mL, 0.38 mol, 1.25 equiv.) was then added to the amino acid solution over a 20 minute period at room temperature. After stirring for 2 hours at room temperature, a precipitate appeared. The precipitate was dissolved by adding 200 mL of additional 2N sodium hydroxide solution. After stirring for an additional 30 minutes, dilute aqueous hydrochloric acid solution (1: 4, H_2O:HCl) was added until the pH of the reaction mixture reached 2.6. A precipitate of modified amino acids formed and was recovered by decantation. This material was dissolved in 2N sodium hydroxide and dried *in vacuo* to give a white solid. Yield of crude modified amino acids = 84.6 g, 77%).

EXAMPLE 7: Modification of Phenylalanine Methyl Ester Using Hippuryl Chloride

L-Phenylalanine Methyl Ester Hydrochloride (15 g, 0.084 mole) was dissolved in dimethylformamide (DMF) (100 mL) and to this was added pyridine (30 mL). A solution of hippuryl chloride (16.6 g, 0084 moles in 100 mL DMF) was immediately added to the amino acid ester solution in two portions. The reaction mixture was stirred at room temperature overnight. The reaction mixture was then reduced *in vacuo* and dissolved in 1N aqueous sodium hydroxide. The solution was heated at 70°C for 3 hours in order to hydrolyze the methyl ester to a free carboxyl group. Thereafter, the solution was acidified to pH 2.25 using dilute aqueous hydrochloric acid solution (1:3 HCl/H_2O). A gum-like precipitate formed and this was recovered and dissolved in 1N sodium hydroxide. The

solution was reduced *in vacuo* to afford 18.6 g of crude modified amino acid product (Yield 18.6 g). After recrystallization from acetonitrile, pure modified phenylalanine (12 g) was recovered as a white powder. m.p. 223-225°C.

Example 8: **Modification of Phenylalanine Using Acetylsalicyloyl Chloride**

10 ml of pyridine were added to a mixture of acetylsalicyloyl chloride (10 g., 50.4 mmol) and phenylalanine benzyl ester toluene sulfonate (21.4 g., 50 mmol). The reaction was allowed to stir overnight and was monitored by thin layer chromatography (TLC). The reaction mixture was washed with 1N hydrochloric acid (100 ml). The organic layer was separated, dried with $MgSO_4$, filtered, and concentrated to provide an oil. The oil was purified by flash chromatography on silica gel (30% EtOAc/hexane) to provide 16.9 g (80.9%) of pure diester product, m.p. 56-57°C, Rf = 0.35 (20% EtOAc/hexane).

The diester, acetylsalicyloyl phenylalanine benzyl ester (30 g. 71.9 mmol), was added to an aqueous saturated solution of $NaHCO_3$ (50 ml), acetone (200 ml), and methanol (125 ml). This mixture was stirred at room temperature and was monitored by TLC until no starting diester remained. Removal of the solvents *in vacuo* provided an oily suspension which was extracted with methylene chloride (3 x 80 ml). The extracts were combined, washed with water, and dried with $MgSO_4$. The dried extracts were filtered and concentrated to provide the benzyl ester product which was purified by flash chromatography on silica gel (30% EtOAc/hexane).

The salicyloyl phenylalanine benzyl ester (16.9 g, 45 mmol) was placed in methanol (400 ml) in a reaction vessel, and a palladium/carbon catalyst was added. The vessel was flushed with nitrogen (3 times), and hydrogen gas (1.7 g) was introduced. The reaction was monitored by TLC and was complete in about 4 hours. The catalyst was removed

by filtration and washed with methanol. Removal of the solvent provided a quantitative yield of the pure salicyloyl phenylalanine, m.p. 52-53°C.

Properties are listed below:

^{1}H NMR(300 MHz, DMSO-d6) δ: 13 (s, 1H), 12 (d, 1H), 9 (d, 1H), 8 (dd, 1H), 7.4 (m, 1H), 7.2 (q, 5H), 6.9 (t, 2H), 4.7 (m, 1H), 3.1 (m, 2H).

Analysis: calculated for $C_{16}H_{15}NO_4$: C,67.36; H,5.30; N,4.91;
Found: C,67.15; H,5.27; N,4.84;

Example 9: Preparation and Evaluation of Desferrioxamine (DFO)-containing Microspheres

In this Example, a study was undertaken to evaluate the relative efficacy of the iron chelator desferrioxamine B (DFO) when administered orally with modified amino acid carriers. Currently, DFO must be administered parenterally in order to treat iron overload in mammals. The currently accepted mode of treatment (subcutaneous infusion of DFO) raises problems of compliance, especially in patients who must be maintained on chelation therapy for long periods of time, and is also difficult to achieve in Third World countries with suboptimal medical facilities. Thus, there is a need for a convenient orally administrable form of DFO. Effective amounts of DFO are well known in the art and range between about 10 and about 50 mg/kg/day (or between about 0.4 to about 2.0 g/day total.)

Preparation of DFO in modified amino acid carriers was performed in the same manner as described above. DFO was obtained from Ciba-Geigy (Basel, Switzerland) under the trade name DESFERAL. DFO modified amino acid carriers were prepared with salicyloyl-Phe modified amino acid, and the final modified amino acid carrier suspension contained 125 mg/ml DFO.

The efficacy of modified amino acid-carried DFO was evaluated in both the Cebus monkey iron clearance model system (Bergeron et al., Sixth Cooley's Anemia Symposium,

Ann.N.Y.Acad.Sci. 612:378 (1991)), and in bile duct-cannulated rats (Bergeron et al., Blood 79:1882 (1992)).

Experimental Procedure

A. Cebus monkeys:

Eight days prior to drug administration, Cebus monkeys were anesthetized with Ketamine (7-10 mg/kg intramuscularly), after which a baseline blood sample was obtained. The animals were then transferred to metabolic cages and started on a low-iron liquid diet. One day prior to administration, the animals were fasted. On day 0, the animals were anesthetized and the drug was administered at a dose of 200 mg/kg using a pill gun. An additional blood sample was obtained on day 5.

Fecal and urine samples were collected at 24-h intervals between day -3 and day +5. The volume of the urine and the wet weight of the feces were determined, after which the samples were autoclaved. The urine samples were then acidified with low-iron nitric acid. The feces samples were freeze-dried for 48h, after which their dry weight was measured; they were then refluxed for 48h with low-iron nitric acid. The iron content of the acidified urine and feces samples was then measured in triplicate on an atomic absorption spectrophotometer. The iron clearance rates were calculated on the basis of a 1:1 DFO-iron complex. The iron output for 4 days prior to administration of the drug was averaged and subtracted from the iron clearance after administration; this value was then divided by the theoretical output to calculate the efficiency of clearance.

Oral administration of DFO in the absence of modified amino acid carriers induced little if any clearance of iron in a 48-h period following administration (Figure 2) In contrast, DFO prepared with the modified amino acid carriers of the present invention prior to administration induced a rapid peak of iron secretion in both urine and feces, corresponding to a 4% clearance rate during that interval. Subcutaneously administered DFO induced a 5%

clearance rate. These results demonstrate that a single dose of DFO modified amino acid carrier was substantially as effective as parenterally administered DFO in delivering a therapeutically active bioavailable dose of DFO.

B. Rats

Male Sprague-Dawley rats were anesthetized, after which their bile ducts were cannulated, such that continuous bile samples could be collected while the animals were free to move around their cages. The rats were fasted for 24h, after which the drug was administered by gavage at a dose of 45 mg/kg. Bile samples were collected at 3-h intervals, and urine samples were taken every 24 h. The iron content of the bile and urine samples were measured by atomic absorption spectrometry essentially as described in part A.

Figure 3 shows the biliary and urinary excretion of iron in rats after oral administration of DFO with modified amino acid carriers. The stimulation of iron excretion by orally administered modified amino acid-carried DFO is equivalent to that observed when free DFO was administered via a subcutaneous route (Bergeron et al., Blood, ibid.).

Example 10: Preparation of Insulin/Delivery System

In a test tube, 1800 mg of salicyloyl phenylalanine carrier were added to 4 ml of water. The solution was stirred, and the pH was adjusted to 8.0-8.5 with NaOH (1.0N) or HCl (1.0N).

Insulin was prepared by adding 20 mg of insulin to 2 mL of $NaHCO_3$ solution (40 mg/ml $NaHCO_3$). The concentration of insulin was 10 mg/ml.

0.900 ml of insulin solution was added to the carrier solution. Water was added to bring the total volume to 6.0 mL. The sample had a carrier concentration of 300 mg/mL. The total insulin concentration was 1.5 mg/mL.

Example 11 **Insulin *In Vivo* Experiments in Rats**

Following the procedure in Example 10, insulin/carrier preparations of 3 mg/kg of insulin mixed with 60 mg/kg of salicyloyl-Phe carrier, 600 mg/kg of salicyloyl-Phe carrier and 1200 mg/kg of salicyloyl-Phe carrier, respectively were prepared. Fasted rats were anesthetized with Ketamine (14 mg/kg). The fasted rats were administered, by oral gavage, a dosing volume of two (2) mL/kg of one of the preparations. Blood samples were collected by cutting the tip of the tail, and one drop of blood was analyzed with a ONE TOUCH II Glucose Analyzer (available from Life Scans, Inc., Mitpitsas, CA, USA).

Results are illustrated in Figure 4.

Example 12 **Insulin *In Vivo* Experiment**

Following the procedure in Example 10, insulin/carrier preparations were prepared, and a first group of fasted rats was administered, by oral gavage, 0.5 mg/kg of insulin mixed with 600 mg/kg of salicyloyl-Phe carrier. A second group of fasted rats was administered 600 mg/kg of salicyloyl-Phe carrier without insulin. Blood samples were collected and analyzed as described in Example 11.

Results are illustrated in Figure 5.

Example 13 **Insulin *In Vivo* Experiment**

Following the procedure in Example 10, insulin/carrier preparations were prepared, and a first group of fasted rats was administered, by oral gavage, 0.5 mg/kg of insulin mixed with 600 mg/kg of salicyloyl-Phe carrier. A second group of fasted rats was administered 3 mg/kg of insulin without a carrier. Blood samples were collected and analyzed as described Example 11.

Results are illustrated in Figure 6.

Example 14 **Insulin *In Vivo* Experiment**

Following the procedure in Example 10, insulin carrier preparations were prepared, and a first group of fasted rats was administered, by oral gavage, 1.0 mg/kg of insulin mixed with 600 mg/kg of salicyloyl-Phe carrier. A second group of fasted rats was administered 600 mg/kg of salicyloyl-Phe carrier without insulin. Blood samples were collected an analyzed as described in Example 11.

Results are illustrated in Figure 7.

Example 15 **Insulin *In Vivo* Experiment**

Following the procedure in Example 10, insulin/carrier preparations were prepared, and a first group of fasted rats was administered, by oral gavage, 3.0 mg/kg of insulin mixed with 1200 mg/kg of salicyloyl-Phe carrier. A second group of fasted rats was administered, by oral gavage, doses of 1200 mg/kg of salicyloyl-Phe carrier without insulin. Blood samples were collected and analyzed as described in Example 11.

Results are illustrated in Figure 8.

Example 16 ***In Vivo* Insulin Experiment**

Following the procedure in Example 10, insulin/carrier preparations were prepared, and a first group of fasted rats was administered, by intraduodenal injection, 1.0 mg/kg of insulin mixed with 300 mg/kg of salicyloyl-Phe carrier. A second group of rats was administered, by intraduodenal injection, 1.0 mg/kg of insulin mixed with 300 mg/kg of salicyloyl-Phe carrier and 6 mg/kg of urea. Blood samples were collected and analyzed as described in Example 11.

Results are illustrated in Figure 9.

Example 17

***In Vivo* Evaluation of Cromoglycate Preparations in Rats**

A preparation of 100 mg/ml salicyloyl-Phe solution in 0.85N citric acid and 0.5% acacia and 25 mg/ml of disodium cromoglycate was prepared.

Fasted rats were administered, by oral gavage, dosages of the preparation containing 50 mg/kg of disodium cromoglycate mixed with 200 mg/kg of carrier, at a dosing volume of two (2) mL/kg. The delivery was evaluated by using the procedure described by A. Yoshimi in *Pharmcobio-Dyn.*, **15**, pages 681-686, (1992).

Results are illustrated in Figure 10.

Example 18

In Vivo Evaluation of Cromoglycate Preparations in Rats

Following the procedure in Example 17, a disodium cromoglycate preparation was prepared, fasted rats were administered, by oral gavage, a mixture of 50 mg/kg of disodium cromoglycate and 400 mg/kg of salicyloyl-Phe carrier, at a dosing volume of two (2) mL/kg.

Results are illustrated in Figure 11.

As clearly illustrated by the data in the Examples and Figures the use of compositions of the subject invention show significant advantages for the delivery of biologically active agents.

WHAT IS CLAIMED IS:

1. A composition of matter comprising (a) an biologically active agent selected from the group consisting of desferrioxamine, insulin and cromolyn sodium; and (b) an acylated amino acid carrier.

2. The composition as defined in claim 1 wherein said biologically active agent is desferrioxamine.

3. The composition as defined in claim 1 wherein said biologically active agent is insulin.

4. The composition as defined in claim 1 wherein said biologically active agent is cromolyn sodium.

5. The composition as defined in claim 1 wherein said acylated amino acid carrier comprises salicyloyl-phenylalanine.

6. The composition as defined in claim 2 wherein said acylated amino acid carrier comprises salicyloyl-phenylalanine.

7. The composition as defined in claim 3 wherein said acylated amino acid carrier comprises salicyloyl-phenylalanine.

8. The composition as defined in claim 4 wherein said acylated amino acid carrier comprises salicyloyl-phenylalanine.

9. A pharmaceutical composition comprising; (a) a biologically active agent selected from the group consisting of desferrioxamine, insulin and cromolyn sodium; (b) an acylated amino acid carrier; and (c) a pharmaceutically acceptable carrier or diluent.

10. The pharmaceutical composition as defined in claim 9 wherein said biologically active agent is desferrioxamine.

11. The pharmaceutical composition as defined in claim 9 wherein said biologically active agent is insulin.

12. The pharmaceutical composition as defined in claim 9 wherein said biologically active agent is cromolyn sodium.

13. The pharmaceutical composition as defined in claim 9 wherein said acylated amino acid carrier comprises salicyloyl-phenylalanine.

14. The pharmaceutical composition as defined in claim 10 wherein said acylated amino acid carrier comprises salicyloyl-phenylalanine.

15. The pharmaceutical composition as defined in claim 11 wherein said acylated amino acid carrier comprises salicyloyl-phenylalanine.

16. The pharmaceutical composition as defined in claim 12 wherein said acylated amino acid carrier comprises salicyloyl-phenylalanine.

17. A method of lowering iron concentration in a mammal comprising orally administering a composition as defined in claim 2.

18. A method of lowering iron concentration in a mammal comprising orally administering a composition as defined in claim 6.

19. A method of treating diabetes in a mammal in need of such treatment comprising orally administering a composition as defined in claim 3.

20. A method of treating diabetes in a mammal in need of such treatment comprising orally administering a composition as defined in claim 7.

21. A method of treating respiratory afflictions in a mammal in need of such treatment comprising orally administering a composition as defined in claim 4.

22. A method of treating respiratory afflictions in a mammal in need of such treatment comprising orally administering a composition as defined in claim 8.

23. A dosage unit form comprising

(A) a pharmacological composition as defined in claim 9; and

(B) (a) an excipient,
(b) a diluent,
(c) a disintegrant,
(d) a lubricant,
(e) a plasticizer,
(f) a colorant,
(g) a dosing vehicle, or
(h) any combination thereof.

FIG. 1

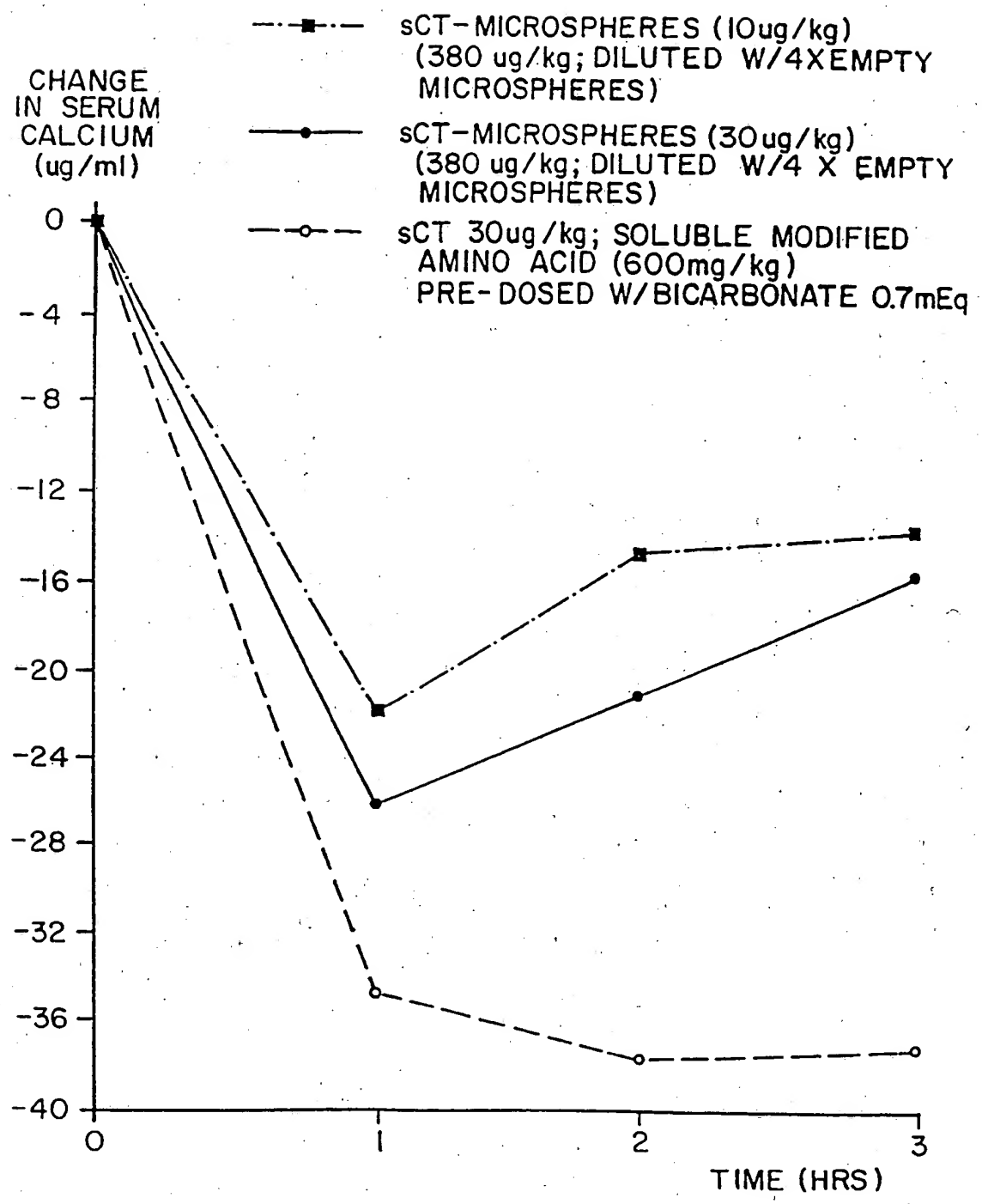

sCT-MICROSPHERES (10ug/kg) (380 ug/kg; DILUTED W/4X EMPTY MICROSPHERES)
sCT-MICROSPHERES (30ug/kg) (380 ug/kg; DILUTED W/4 X EMPTY MICROSPHERES)
sCT 30ug/kg; SOLUBLE MODIFIED AMINO ACID (600mg/kg) PRE-DOSED W/BICARBONATE 0.7mEq
CHANGE IN SERUM CALCIUM (ug/ml)
0
-4
-8
-12
-16
-20
-24
-28
-32
-36
-40
0
1
2
3
TIME (HRS)

FIG. 2

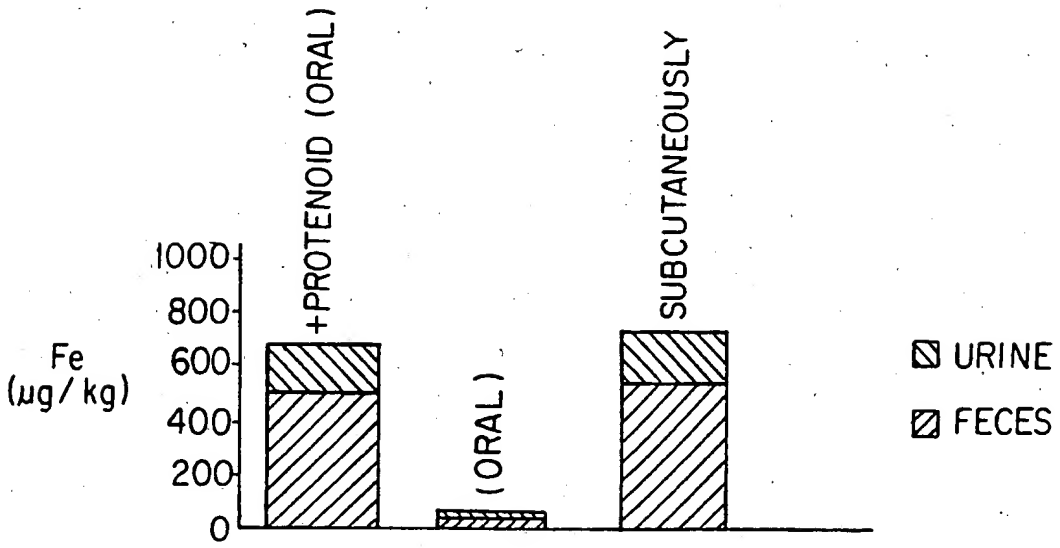

+PROTENOID (ORAL)
(ORAL)
SUBCUTANEOUSLY
Fe
(µg/kg)
1000
800
600
400
200
0
URINE
FECES

FIG. 3

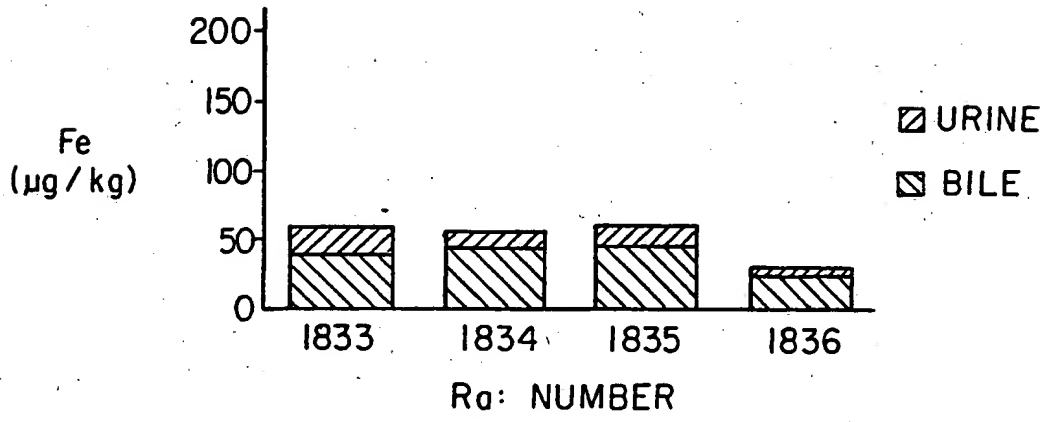

Fe
(µg/kg)
200
150
100
50
0
1833
1834
1835
1836
Ra: NUMBER
URINE
BILE

FIG. 4

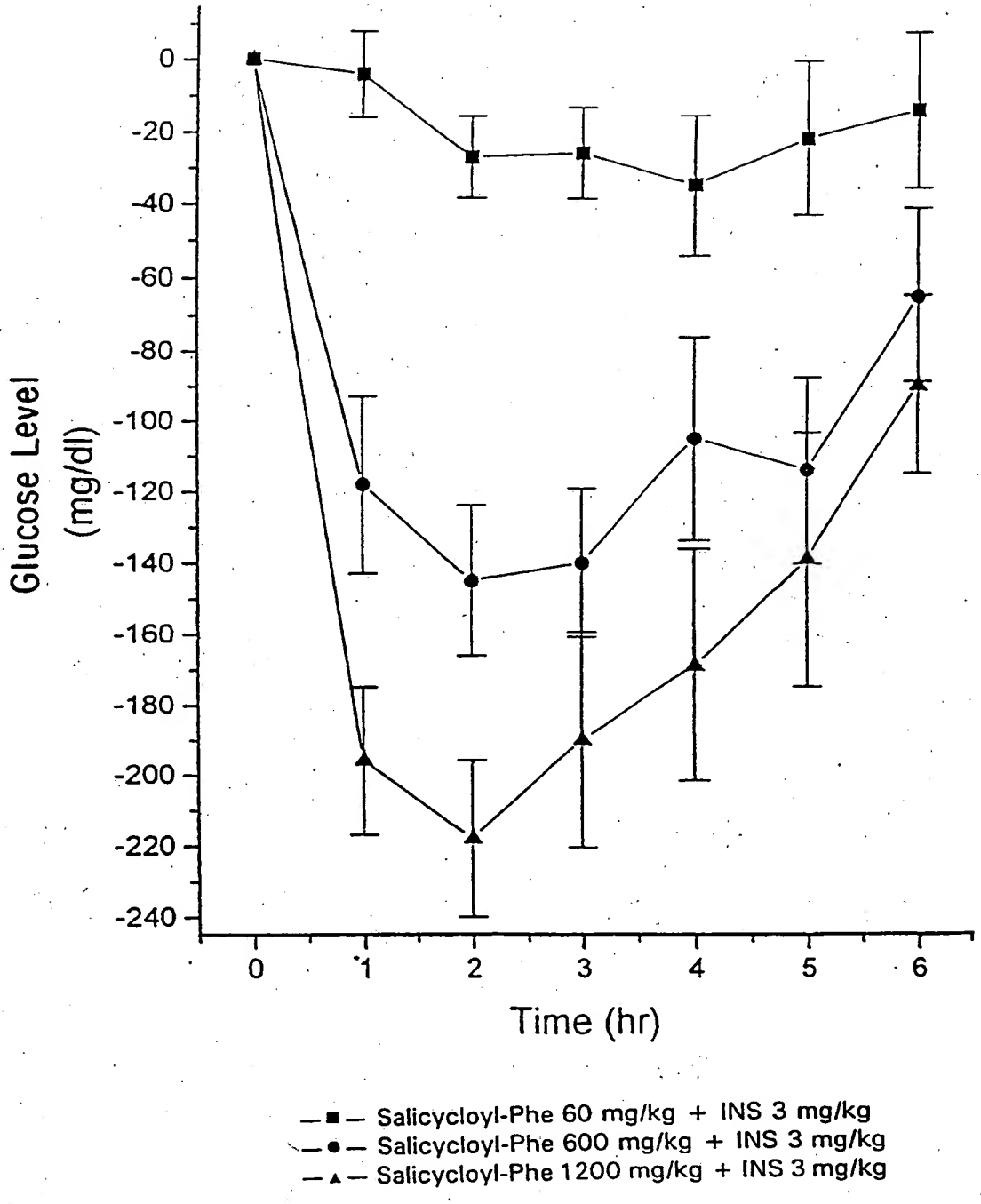

Glucose Level
(mg/dl)
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
0
1
2
3
4
5
6
Time (hr)
— ■ — Salicycloyl-Phe 60 mg/kg + INS 3 mg/kg
— ● — Salicycloyl-Phe 600 mg/kg + INS 3 mg/kg
— ▲ — Salicycloyl-Phe 1200 mg/kg + INS 3 mg/kg

FIG. 5

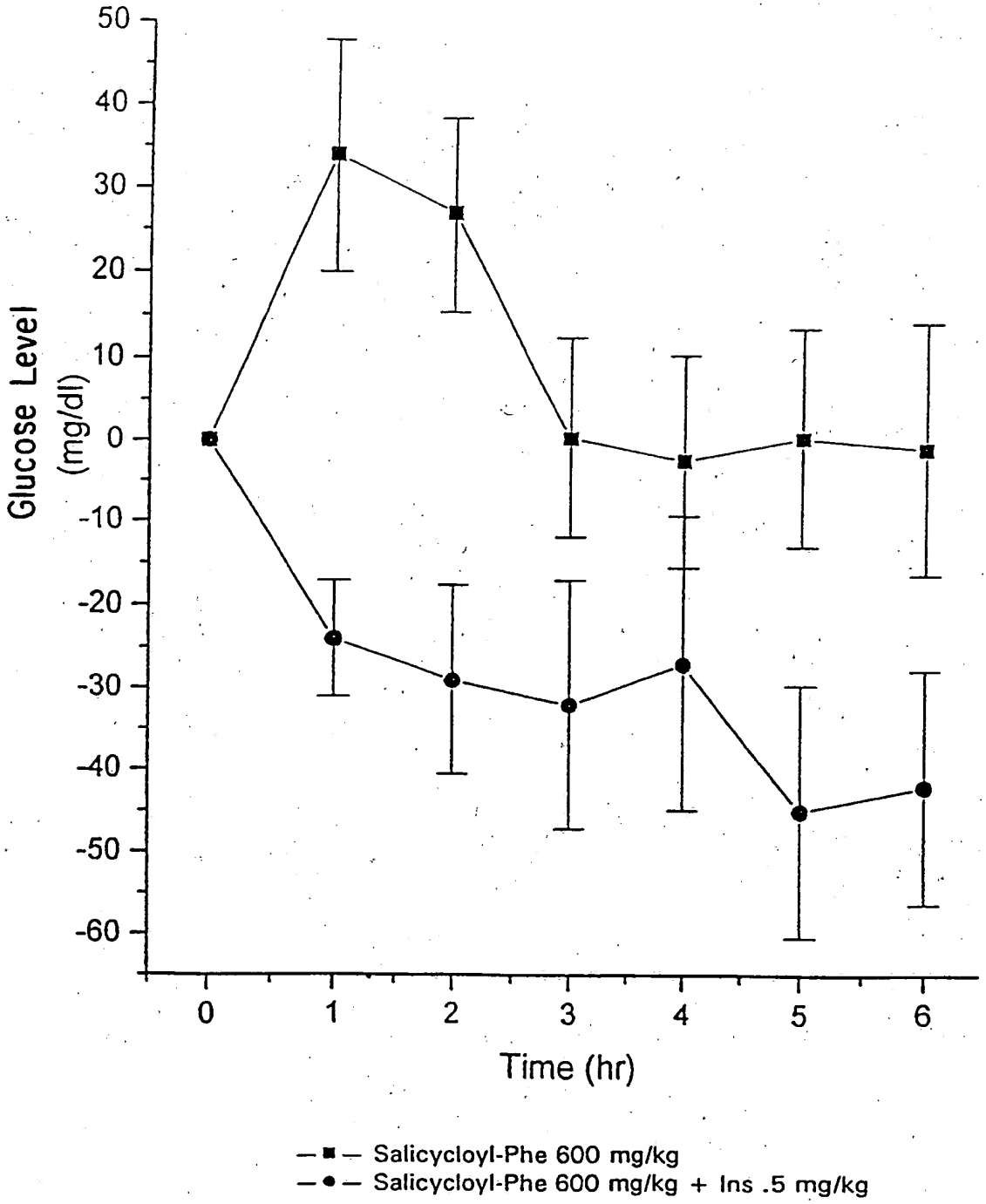

Glucose Level (mg/dl)
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
0
1
2
3
4
5
6
Time (hr)
— ■ — Salicycloyl-Phe 600 mg/kg
— ● — Salicycloyl-Phe 600 mg/kg + Ins .5 mg/kg

FIG. 6

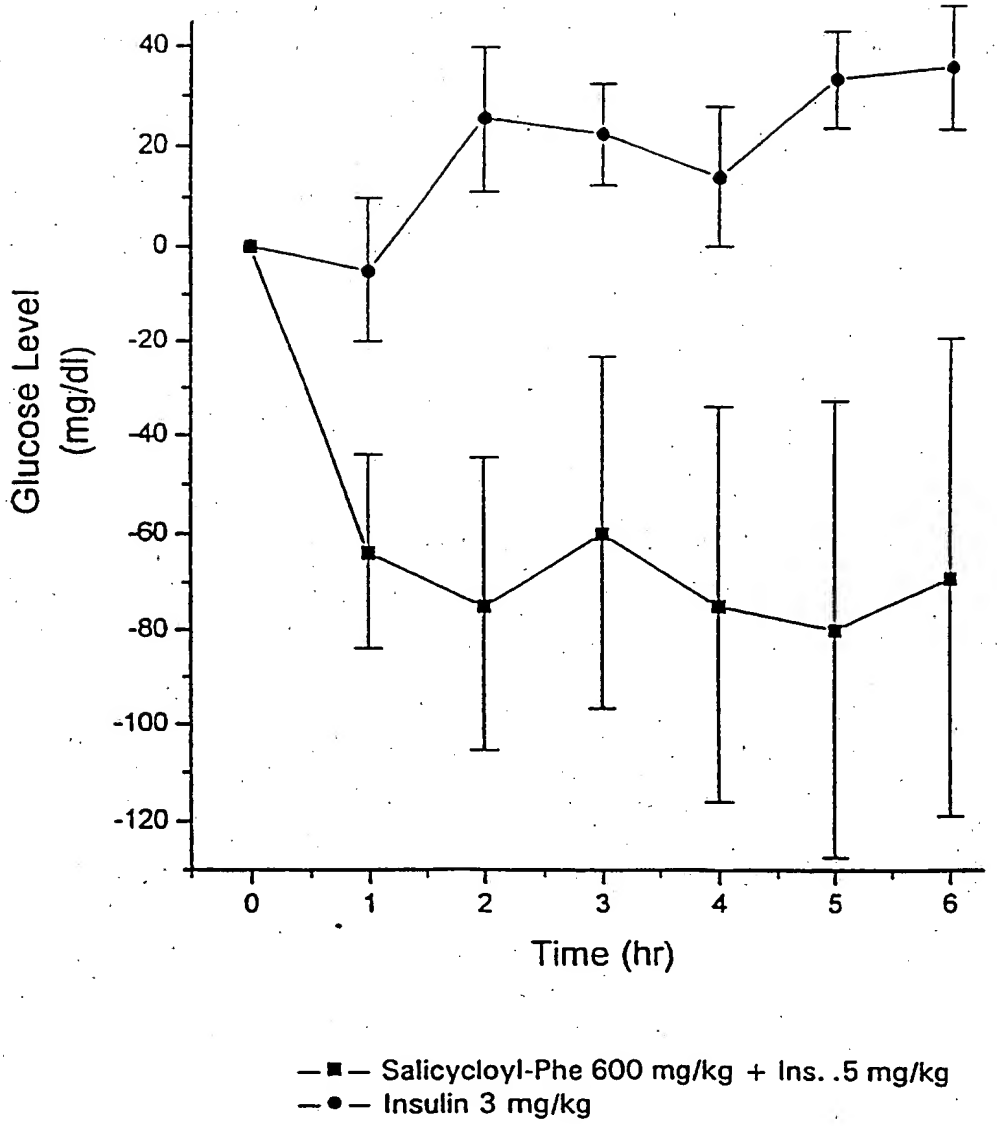

Glucose Level (mg/dl)
40
20
0
-20
-40
-60
-80
-100
-120
0
1
2
3
4
5
6
Time (hr)
—■— Salicycloyl-Phe 600 mg/kg + Ins. .5 mg/kg
—●— Insulin 3 mg/kg

FIG. 7

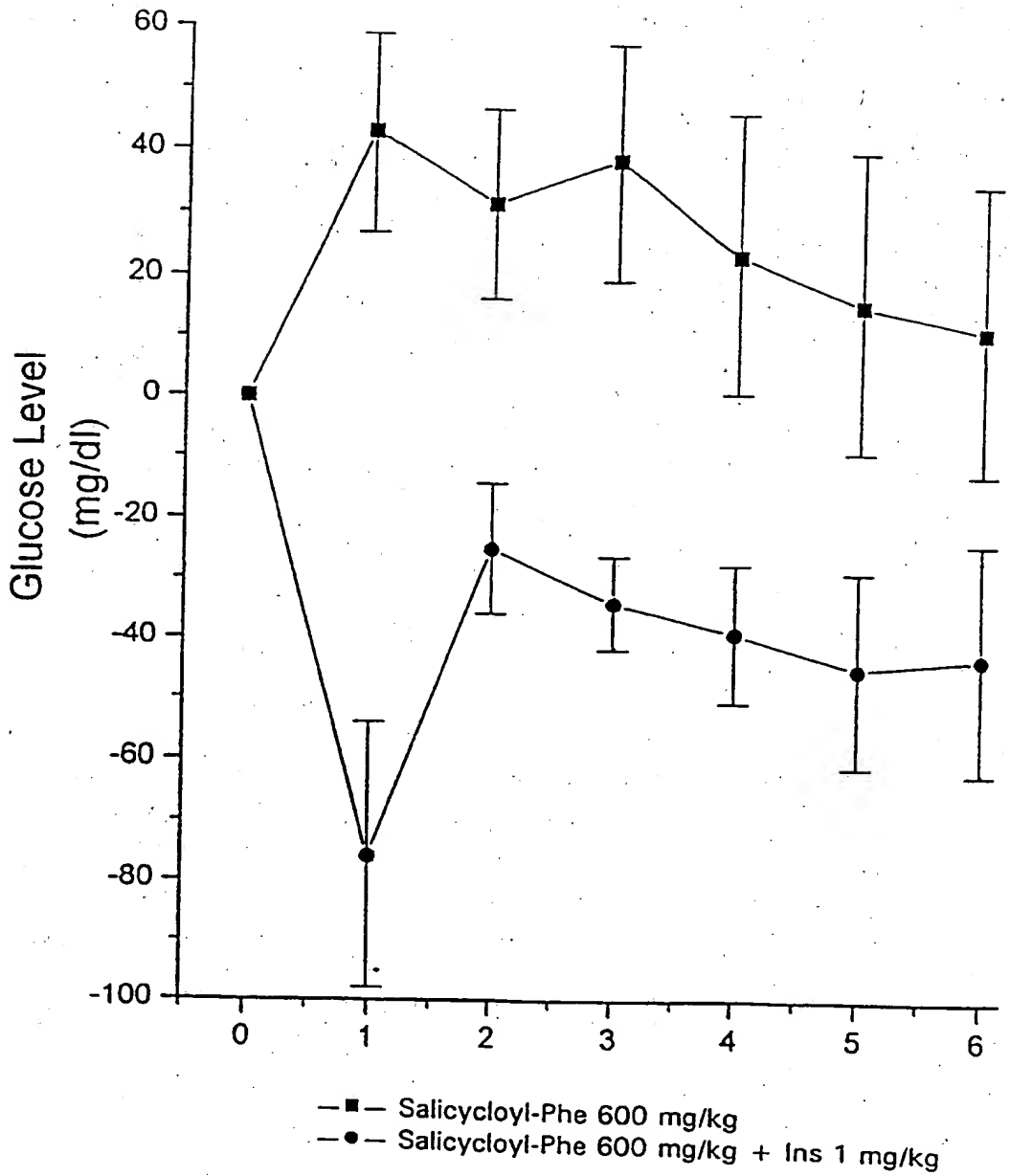

60
40
20
0
-20
-40
-60
-80
-100
Glucose Level (mg/dl)
0
1
2
3
4
5
6
—■— Salicycloyl-Phe 600 mg/kg
—●— Salicycloyl-Phe 600 mg/kg + Ins 1 mg/kg

FIG. 8

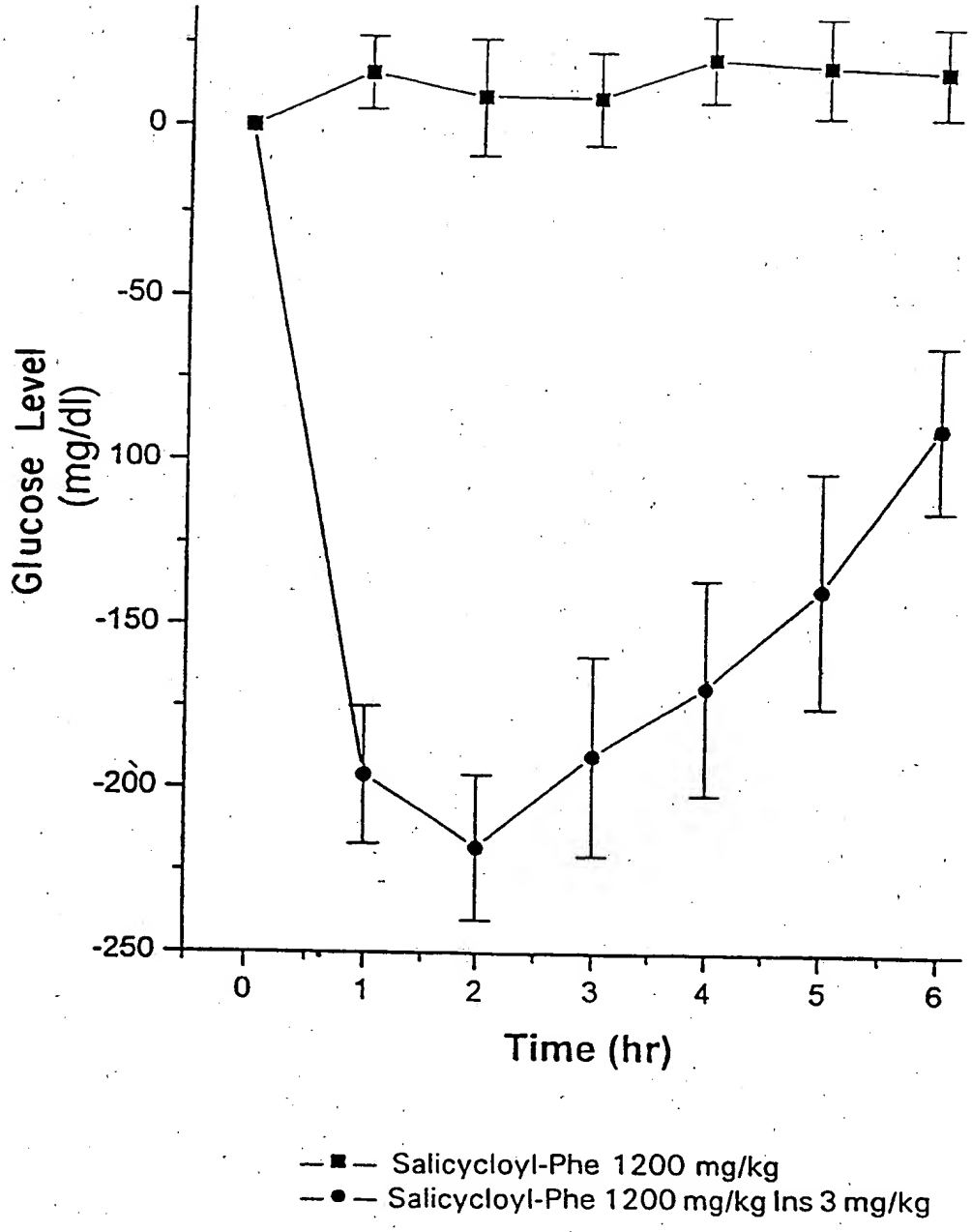

Glucose Level (mg/dl)
0
-50
-100
-150
-200
-250
0
1
2
3
4
5
6
Time (hr)
— ■ — Salicycloyl-Phe 1200 mg/kg
— ● — Salicycloyl-Phe 1200 mg/kg Ins 3 mg/kg

FIG. 9

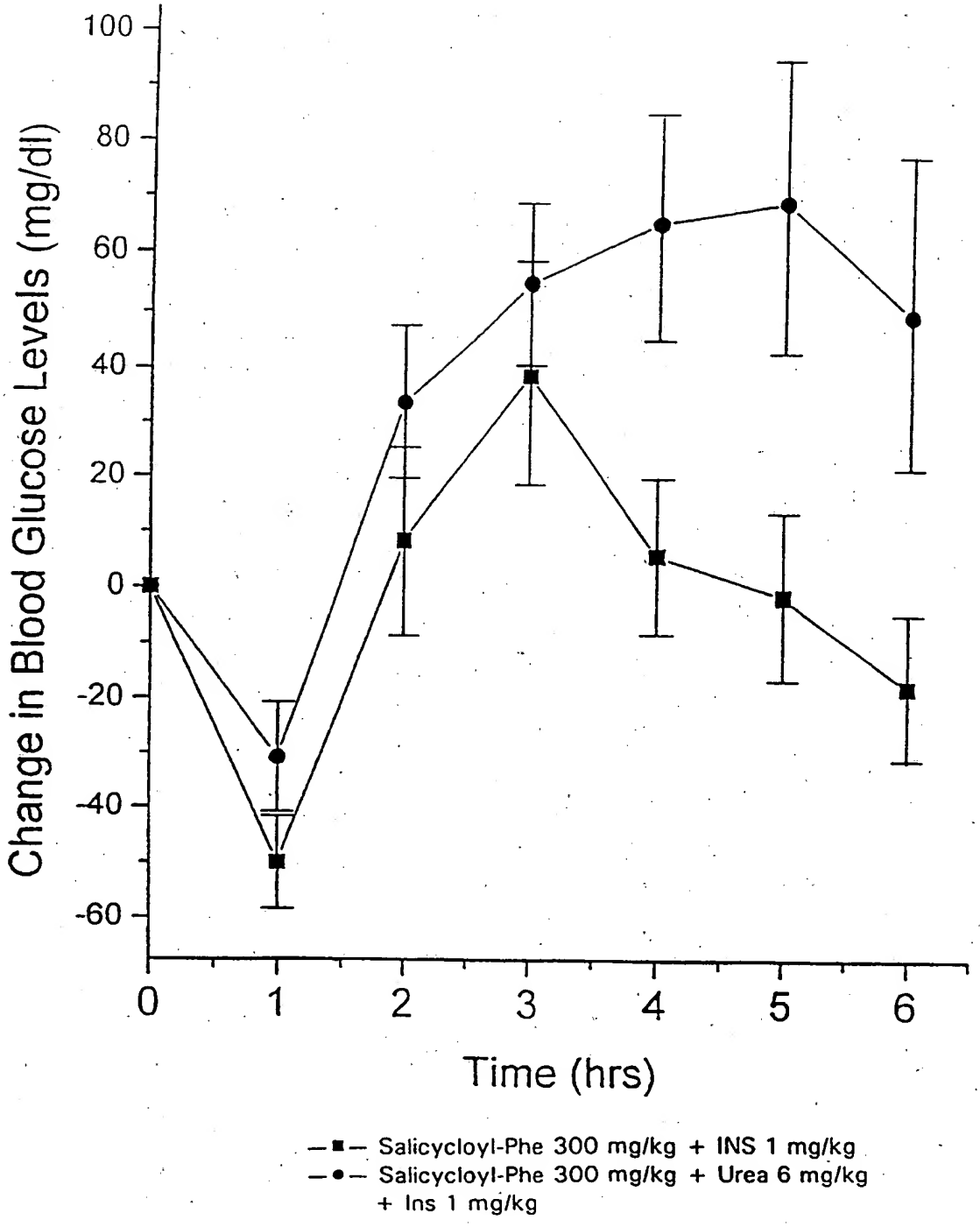

Change in Blood Glucose Levels (mg/dl)
100
80
60
40
20
0
-20
-40
-60
0
1
2
3
4
5
6
Time (hrs)
—■— Salicycloyl-Phe 300 mg/kg + INS 1 mg/kg
—●— Salicycloyl-Phe 300 mg/kg + Urea 6 mg/kg + Ins 1 mg/kg

FIG. 10

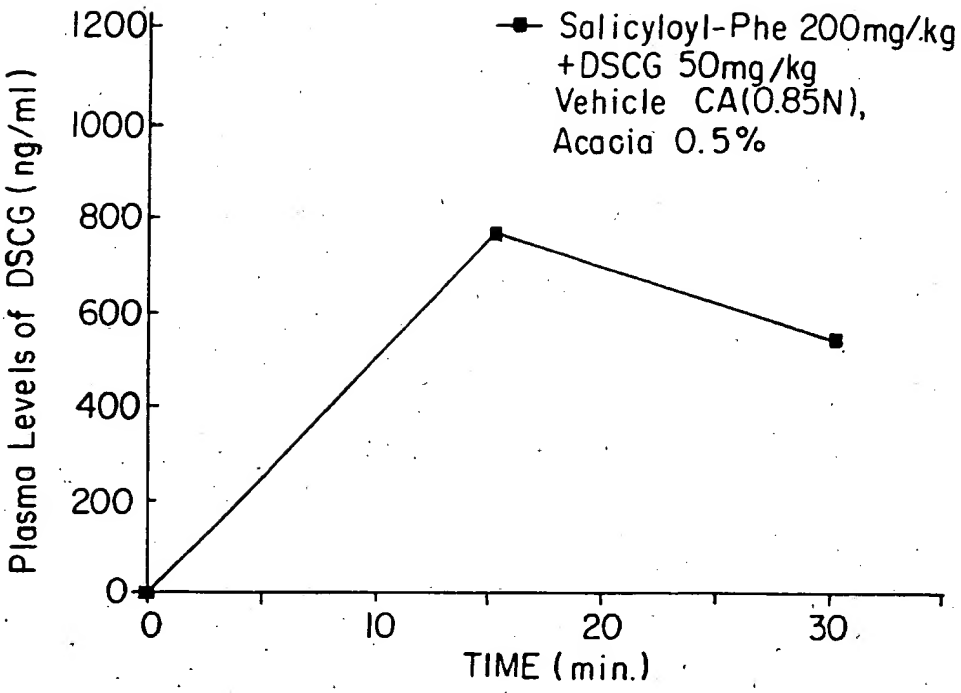
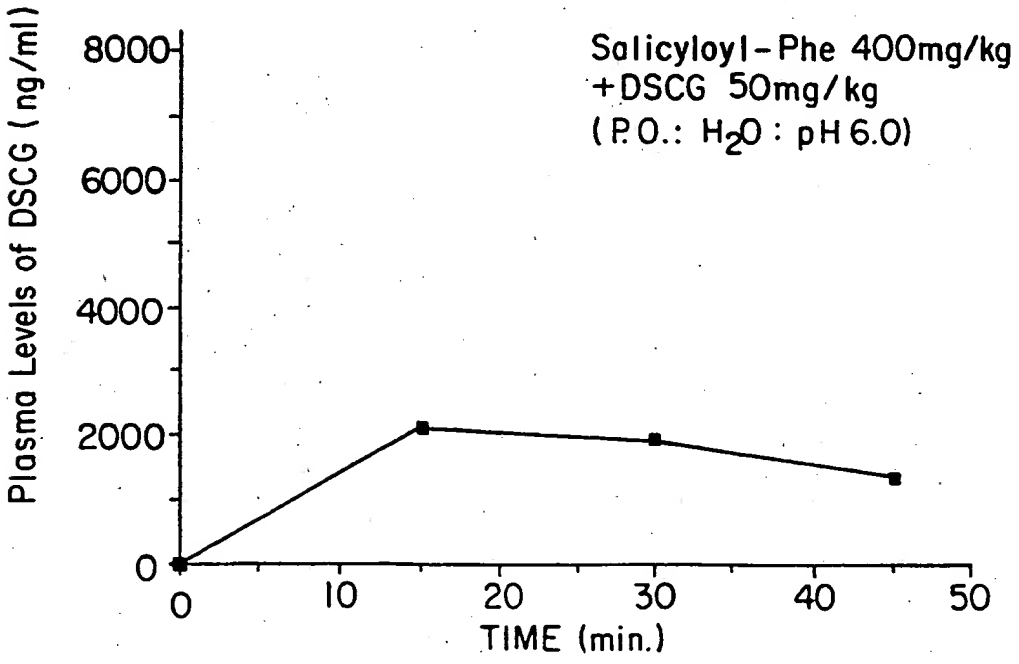

Salicyloyl-Phe 200mg/kg
+DSCG 50mg/kg
Vehicle CA(0.85N),
Acacia 0.5%
Plasma Levels of DSCG (ng/ml)
1200
1000
800
600
400
200
0
0
10
20
30
TIME (min.)

FIG. 11


Salicyloyl-Phe 400mg/kg
+DSCG 50mg/kg
(P.O.: H2O : pH 6.0)
Plasma Levels of DSCG (ng/ml)
8000
6000
4000
2000
0
0
10
20
30
40
50
TIME (min.)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12333

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : A61K 37/00; A01N 43/16
US CL :514/12, 456
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 456

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Pediatric Research, Volume 23, Number 4, Part 2, issued 1988, Watterberg et al., "Aerosolized cromolyn sodium delivery to intubated babies", page 570A, column 1, abstract number 2209, see entire abstract.	1, 4, 5, 8, 9, 12, 13, 16, 21-23
Y	Chest, Volume 87, Number 1 (Supplement), issued 1985, Bernstein, "Cromolyn Sodium", pages 68S-73S, see entire article.	1, 4, 5, 8, 9, 12, 13, 16, 21-23
Y	Diabetes, Volume 37, issued February 1988, Damge et al., "New approach for oral administration of insulin with polyalkylcyanoacrylate nanocapsules as drug carrier", pages 246-251, see entire article	1, 3, 5, 7, 9, 11, 13, 15, 19, 20, 23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 JANUARY 1995	FEB 03 - 1995

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	BENET PRICKRIL
Facsimile No. (703) 305-3230	Telephone No. (703) 305-9646

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12333

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Intern...onal application No.
PCT/US94/12333

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claims 1, 2, 5, 6, 9, 10, 13, 14, 17, 18, and 23, drawn to compositions containing desferrioxamine and methods of lowering iron concentration in a mammal.
Group II, claims 1, 3, 5, 7, 9, 11, 13, 15, 19, 20, and 23, drawn to compositions containing insulin and methods of treating diabetes in a mammal in need thereof.
Group III, claims 1, 4, 5, 8, 9, 12, 13, 16, 21, 22, and 23, drawn to compositions containing cromolyn sodium and methods of treating respiratory afflictions in a mammal in need thereof.

The following claims are generic: Claims 1, 5, 9, 13, and 23.
The inventions listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Groups I, II, and III are distinct chemically and structurally, and as a whole these groups do not define a single contribution over the prior art. Insulin is a well known glucose-regulating protein, desferrioxamine is a natural product useful as an iron chelator, and cromolyn sodium is a heterocycle useful in treating bronchial asthma. The large differences between these species relating to both their chemical or physical distinctness and their disparate utility is the basis for holding that they do not relate to a single inventive concept. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to define a single general inventive concept.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or correspondingspecialtechnicalfeatures for the following reasons: The three species listed as biologically active agents (desferrioxamine, insulin, and cromolyn sodium) are widely disparate in their chemical, structural, and biological properties and clearly lack the same or corresponding technical features.

1/3/1
DIALOG(R)File 345:Inpadoc/Fam.& Legal Stat

15174898
Basic Patent (No,Kind,Date): IL 106015 A0 19931020 <No. of Patents: 120>
PROTEINOID CARRIERS AND METHODS FOR PREPARATION AND USE (English)
Patent Assignee: EMISPHERE TECHNOLOGIES INC
IPC: *C07K;
Language of Document: English
Patent Family:

Patent No	Kind	Date	Applic No	Kind	Date	
AU 9346356	A1	19940104	AU 9346356	A	19930615	
AU 9468192	A1	19941108	AU 9468192	A	19940422	
AU 9471082	A1	19950103	AU 9471082	A	19940614	
AU 9480936	A1	19950522	AU 9480936	A	19941024	
AU 9523644	A1	19951116	AU 9523644	A	19950421	
AU 9523963	A1	19951116	AU 9523963	A	19950421	
AU 9539633	A1	19960515	AU 9539633	A	19951016	
AU 9540052	A1	19960515	AU 9540052	A	19951016	
AU 9641292	A1	19960426	AU 9641292	A	19950928	
AU 9641293	A1	19960419	AU 9641293	A	19950928	
AU 9641524	A1	19960515	AU 9641524	A	19951024	
AU 9651315	A1	19960731	AU 9651315	A	19960116	
AU 9855322	A1	19980703	AU 9855322	A	19971209	
AU 697044	B2	19980924	AU 9471082	A	19940614	
AU 704776	B2	19990506	AU 9651315	A	19960116	
AU 711887	B2	19991021	AU 9539633	A	19951016	
BR 9306678	A	19981208	BR 93U6678	A	19930615	
BR 9510168	A	19971014	BR 95U10168	A	19951016	
CA 2160693	AA	19941027	CA 2160693	A	19940422	
CA 2164957	AA	19941222	CA 2164957	A	19940614	
CA 2174961	AA	19950504	CA 2174961	A	19941024	
CA 2188467	AA	19951102	CA 2188467	A	19950421	
CA 2202299	AA	19960502	CA 2202299	A	19951016	
CA 2203033	AA	19960502	CA 2203033	A	19951016	
CA 2209492	AA	19960718	CA 2209492	A	19960116	
EP 642532	A1	19950315	EP 93916542	A	19930615	
EP 696208	A1	19960214	EP 94916578	A	19940422	
EP 706375	A1	19960417	EP 94920205	A	19940614	
EP 726771	A1	19960821	EP 94932077	A	19941024	
EP 758843	A1	19970226	EP 95917157	A	19950421	
EP 781124	A1	19970702	EP 95939863	A	19951024	
EP 783299	A1	19970716	EP 95937558	A	19951016	
EP 784469	A1	19970723	EP 95938814	A	19951016	
EP 812212	A1	19971217	EP 96907856	A	19960116	
EP 1025840	A2	20000809	EP 2000103527	A	19940422	
EP 1072255	A2	20010131	EP 2000117452	A	19941024	
EP 1077070	A2	20010221	EP 2000118505	A	19940422	
EP 1025840	A3	20000830	EP 2000103527	A	19940422	
EP 696208	A4	19970129	EP 94916578	A	19940422	
EP 706375	A4	19961106	EP 94920205	A	19940614	
EP 726771	A4	19970129	EP 94932077	A	19941024	
EP 758843	A4	19980520	EP 95917157	A	19950421	
EP 783299	A4	19990630	EP 95937558	A	19951016	
EP 784469	A4	19990324	EP 95938814	A	19951016	
EP 812212	A4	19990414	EP 96907856	A	19960116	
FI 9405912	A	19950215	FI 945912	A	19941215	
FI 9701776	A	19970425	FI 971776	A	19970425	
FI 9702945	A	19970711	FI 972945	A	19970711	
FI 9405912	A0	19941215	FI 945912	A	19941215	
FI 9701776	A0	19970425	FI 971776	A	19970425	
FI 9702945	A0	19970711	FI 972945	A	19970711	
HU 9702307	AB	19980330	HU 979702307	A	19960116	
HU 9403589	A0	19950228	HU 949403589	A	19930615	
HU T70211	A2	19950928	HU 949403589	A	19930615	
HU T77759	A2	19980728	HU 989800903	A	19951016	
IL 106015	A0	19931020	IL 106015	A	19930614	(BASIC)
IL 109403	A0	19940731	IL 109403	A	19940422	

IL 111385	A0	19941229	IL 111385	A	19941024
IL 113455	A0	19950731	IL 113455	A	19950420
IL 113456	A0	19950731	IL 113456	A	19950420
JP 7508004	T2	19950907	JP 93501793	A	19930615
JP 8509474	T2	19961008	JP 94523595	A	19940422
JP 8511545	T2	19961203	JP 94502192	A	19940614
JP 9504300	T2	19970428	JP 95512827	A	19941024
JP 9512279	T2	19971209	JP 95527834	A	19950421
JP 10507762	T2	19980728	JP 95514062	A	19951016
JP 10509432	T2	19980914	JP 95514063	A	19951016
JP 10509433	T2	19980914	JP 95514159	A	19951024
JP 11514331	T2	19991207	JP 96521879	A	19960116
NO 9404852	A	19950210	NO 944852	A	19941214
NO 9701889	A	19970623	NO 971889	A	19970424
NO 9703250	A	19970905	NO 973250	A	19970711
NO 9404852	A0	19941214	NO 944852	A	19941214
NO 9701889	A0	19970424	NO 971889	A	19970424
NO 9703250	A0	19970711	NO 973250	A	19970711
PL 319833	A1	19970901	PL 319833	A	19951016
PL 321275	A1	19971124	PL 321275	A	19960116
US 5443841	A	19950822	US 920346	A	19920727
US 5447728	A	19950905	US 168776	A	19931216
US 5451410	A	19950919	US 51019	A	19930422
US 5541155	A	19960730	US 231623	A	19940422
US 5578323	A	19961126	US 76803	A	19930614
US 5601846	A	19970211	US 437698	A	19950509
US 5629020	A	19970513	US 231622	A	19940422
US 5643957	A	19970701	US 335148	A	19941025
US 5693338	A	19971202	US 315200	A	19940929
US 5709861	A	19980120	US 372208	A	19950113
US 5714167	A	19980203	US 328932	A	19941025
US 5766633	A	19980616	US 537888	A	19951023
US 5792451	A	19980811	US 205511	A	19940302
US 5811127	A	19980922	US 635921	A	19960424
US 5840340	A	19981124	US 705808	A	19960830
US 5907328	A	19990525	US 920346	A	19970827
US 5935601	A	19990810	US 732404	A	19961022
US 5955503	A	19990921	US 795833	A	19970206
US 5958457	A	19990928	US 438644	A	19950510
US 5976569	A	19991102	US 841101	A	19970429
US 6071538	A	20000606	US 940056	A	19970930
US 6099856	A	20000808	US 763183	A	19961210
US 6100298	A	20000808	US 795837	A	19970206
US 6180140	BA	20010130	US 460265	A	19950602
WO 9423767	A1	19941027	WO 94US4560	A	19940422
WO 9428878	A1	19941222	WO 94US6735	A	19940614
WO 9511690	A1	19950504	WO 94US12333	A	19941024
WO 9528838	A1	19951102	WO 95US5112	A	19950421
WO 9528920	A1	19951102	WO 95US5110	A	19950421
WO 9609813	A1	19960404	WO 95US12888	A	19950928
WO 9610396	A1	19960411	WO 95US12887	A	19950928
WO 9612473	A1	19960502	WO 95US13527	A	19951016
WO 9612474	A1	19960502	WO 95US13528	A	19951016
WO 9612475	A1	19960502	WO 95US14598	A	19951024
WO 9621464	A1	19960718	WO 96US871	A	19960116
WO 9825589	A1	19980618	WO 97US23545	A	19971209
WO 9325583	A2	19931223	WO 93US5723	A	19930615
WO 9325583	A3	19940804	WO 93US5723	A	19930615
ZA 9402804	A	19950104	ZA 949402804	A	19940422
ZA 9408342	A	19950622	ZA 949408342	A	19941024
ZA 9503246	A	19960112	ZA 953246	A	19950421
CZ 9403154	A3	19960117	CZ 943154	A	19930615
CZ 9701229	A3	19971015	CZ 971229	A	19951016

Priority Data (No,Kind,Date):

WO 93US5723 A 19930615
US 898909 A 19920615
US 920346 A 19920727
US 76803 A 19930614
US 51019 A 19930422

US 205511 A 19940302
WO 94US4560 W 19940422
WO 94US6735 W 19940614
US 143571 A 19931026
US 168776 A 19931216
WO 94US12333 W 19941024
US 231623 A 19940422
WO 95US5110 W 19950421
US 231622 A 19940422
WO 95US5112 W 19950421
US 335148 A 19941025
WO 95US13527 W 19951016
US 335147 A 19941025
US 438644 A 19950510
WO 95US13528 W 19951016
US 315200 A 19940929
WO 95US12888 W 19950928
US 316404 A 19940930
WO 95US12887 W 19950928
US 328932 A 19941025
WO 95US14598 W 19951024
US 372208 A 19950113
WO 96US871 W 19960116
US 763183 A 19961210
WO 97US23545 W 19971209
WO 93US5723 W 19930615
EP 94916578 A3 19940422
EP 94932077 A3 19941024
US 898909 B2 19920615
US 51019 A2 19930422
US 143571 B2 19931026
US 76803 A2 19930614
US 920346 A2 19920727
US 437698 A 19950509
US 920346 A3 19920727
US 231622 A2 19940422
US 205511 A2 19940302
US 231623 A2 19940422
US 315200 A2 19940929
US 316404 A2 19940930
US 168776 A2 19931216
US 537888 A 19951023
US 635921 A 19960424
US 705808 A 19960830
US 76803 A3 19930614
US 920346 A 19970827
US 732404 A 19961022
US 231622 A1 19940422
US 795833 A 19970206
US 335148 A3 19941025
WO 94US4560 A2 19940422
US 335147 B2 19941025
US 841101 A 19970429
US 315200 A1 19940929
US 940056 A 19970930
US 328932 A1 19941025
US 920346 A2 19970827
US 328932 A2 19941025
US 795837 A 19970206
US 460265 A 19950602
US 231622 A3 19940422
US 372208 A1 19950113
US 898909 A2 19920615
US 93@@URNISHED A2 19930614

?